REMARKS

Further and favorable reconsideration is respectfully requested in view of the foregoing amendment and following remarks.

Upon entry of the foregoing amendment, claims 1-22 are pending. Claims 1-7 have been amended. Claims 9-22 have been added.

Applicants respectfully request the Examiner to indicate acknowledgment of the perfection of foreign priority in the next Office Action Summary Sheet. Applicants note that the notification of acceptance of the application under 35 U.S.C. §371 (Form PCT/DO/EO/903) clearly indicates receipt of the priority document by the USPTO.

For the Examiner's convenience, a copy of PCT/IB/304 form has been attached to this response as evidence that the International Bureau has received a certified copy of the priority document.

In response to item 2 on page 2 of the Office Action wherein the Examiner indicated not considering references cited in the PCT international search report since they were not in the English language, Applicants note that MPEP §609 A(3) (page 600-122 of the eighth edition, August 2001) expressly states that "**where the information listed is not in the English language, but was cited in a search report ... by a foreign patent office in a counterpart foreign application, the requirement for a concise explanation of relevance can be satisfied by submitting an English-language version of the search report ... indicat[ing] the degree of relevance** .... This application is a 35 U.S.C. 371 and a copy of the PCT international search report, in the English language indicating the degree of relevance of these references, was provided to the Examiner with the IDS filed March 13, 2000.

However, in the interest of furthering prosecution, Applicants have attached hereto Derwent and JPO abstracts of the AK reference. The AJ reference corresponds to WO93/24531 which is a publication in the English language previously submitted with the Supplemental IDS of July 11, 2000. In addition, the Applicants are also submitting with this response a copy of the English translation of the Japanese priority document (JP 246684/1997).

The Amendment

The specification has been amended to provide the appropriate cross-reference to the international parent application to conform with U.S. patent practice.

No new matter has been added to the specification.

Claims 1-3 and 6 have been amended in response to the 35 U.S.C. §112, second paragraph rejection in item 3 on page 2 of the Office Action. The phrase "characterized in" has been removed from claims 1 and 6 and replaced with "wherein" and "comprising" respectively. In addition, the sequence ID NO. in claims 2 and 3 has been changed from the nucleic acid SEQ ID NO: 1 to the amino acid SEQ ID NO: 2 to conform with the recited limitations in the claims.

New claims 9 and 10 recite the immunoassay containing a γ -BNP derivative comprising the nucleic acid sequence of SEQ ID NO: 1 and the second antibody reactive therewith, respectively.

Support for new claims 11-13 may be found on page 8, lines 3-17 of the present specification and in original claims 1-7. Support for new claims 14-16 may be found on page 7, lines 20-24 and on page 8, lines 8-17. Support for new claims 17-22 may be found on page 7, line 25 to page 8, line 17 and in Example 1 of the specification.

In addition, claims 1-5 and 7 have been further amended for editorial purposes and such amendments do not narrow the claimed scope of subject matter.

No new matter has been added to the claims.

Please find enclosed a marked-up version of the specification and of the claims entitled "Version with Markings to Show Changes Made".

Please also find attached a corrected sequence listing removing two typographical errors in the labeling of the amino acids. No new matter has been added to the sequence listing.

Response to the 35 U.S.C. §112 Rejection

In item 3 on page 2 of the Office Action, claims 1-8 were rejected under 35 U.S.C. 112, second paragraph as indefinite.

The Examiner objected to the phrase "characterized in" in claims 1 and 6 as being unclear. Applicants have amended the claims to delete the phrase "characterized in" and replace it with "wherein" in claim 1 and "comprising" in claim 6 respectively which are more acceptable terms in U.S. patent practice.

The Examiner noted that claims 1-3 recite amino acid sequences whereas SEQ ID NO. 1 in claim 1 is a nucleic acid sequence. Applicants have thus amended claims 1-3 to recite SEQ ID NO. 2, which is the amino acid sequence, instead of SEQ ID NO. 1, which is a nucleic acid base sequence.

Therefore, the instant rejection of record has become moot.

Response to the 35 U.S.C. §102 Rejection

In item 4 on page 2, claims 1-5 were rejected under 35 U.S.C. §102(b) over Hunt et al. (Biochemical and Biophysical Research Comm., Vol. 214, No. 3, pages 1175 -83). Applicants respectfully traverse this rejection.

Brief Summary of the Invention

Before addressing this rejection, Applicants believe that it would be beneficial to describe the claimed invention.

The present invention relates to an immunoassay for the brain natriuretic peptide (BNP) which is a member of the natriuretic peptide family. Particularly, this invention relates to an immunoassay for γ-BNP and derivatives thereof.

Various heart diseases stimulate the secretion of cardiac hormones which reflect change in cardiac functions. The secretion of atrial natriuretic peptide (ANP) is accelerated when the *atrium* undergoes a load, whereas the biosynthesis and secretion of BNP is stimulated when the *ventricle* undergoes a load. Thus, both ANP and BNP are useful indicators in the diagnosis of heart disease. In fact, BNP level in patients with heart failure may increase from ten to several hundred times of that of healthy subjects. This change in BNP levels in patients with heart failure is so prominent that detection of BNP levels are an incomparable indicator of cardiac failure.

The present inventors have found an accurate method of diagnosing cardiac disease involving determination of BNP levels by inventing an immunoassay which is specific for mammalian γ-BNP derivatives structurally comprising the α-BNP moiety but not in the form of α-BNP, which until now had been considered the dominant form. The inventors have found that γ-BNP is more stable than α-BNP in blood thus establishing an inventive method specific for γ-BNP to accomplish an accurate diagnosis of cardiac diseases.

For the Examiner's convenience, Applicants have attached to this response Appendix I depicting the relationship between prepro-BNP, pro-BNP (γ-BNP) and γ-BNP derivatives in terms of their respective amino acid sequences. Appendix I clearly shows that the ***carboxyl terminal*** (77-108) of γ-BNP and also of γ-BNP derivatives structurally comprise an active form of α-BNP. Accordingly, the immunoassay of the present invention specifically allows for the determination of γ-BNP derivative levels, without measuring α-BNP, which leads to a clinically significant, convenient and more stable means for the diagnosis and prognostic monitoring of heart failure distinct from conventional BNP assays.

As recited in claim 1, the present invention is drawn to an immunoassay which is specific for *mammalian γ-BNP derivatives*. Mammalian γ-BNP derivatives include "mammalian γ-BNP" that is a pro-BNP comprising 32 amino acids corresponding to α-BNP as a partial peptide at the carboxyl terminal region, as well as peptide fragments derived from mammalian prepro-BNP or γ-BNP through mainly the *in vivo* protease reaction, which fragment is larger than α-BNP. It is noted again that an immunoassay of the present invention is specific for mammalian γ-BNP derivatives, and such derivatives structurally comprise the α-BNP moiety. (See Appendix I)

The Hunt et al. Reference

Hunt et al. discloses a radio immunoassay to an N-terminal pro-BNP fragment [BNP(1-76)], as described in the second and third paragraphs on page 1176. That fragment is referred to as pro-BNP(1-76), which is prepro-BNP(27-102). As shown in Appendix I, the fragment does not structurally comprise the α-BNP moiety and is thus is not specific to γ-BNP derivatives.

Comparison Between the Present Invention and the Hunt et al. Reference

In the instant rejection, the Examiner refers to Table 1 in Hunt et al. However, Table 1 merely shows plasma concentrations of each of ir-N-terminal pro-BNP and ir-BNP-32. The ir-N-terminal pro-BNP is determined using an antibody to pro-BNP(1-13), also referred to as prepro-BNP(27-39), as described in the "Materials and Methods" section on page 1176. This method detects **all** compounds having the peptide sequence of prepro-BNP(27-39), which compounds do not only encompass the N-terminal pro-BNP, prepro-BNP, and pro-BNP but also peptide fragments derived from these BNPs through mainly the in vivo protease reaction of their carboxyl terminals. **Such peptide fragments do not structurally comprise the α-BNP moiety.**

On the other hand, the present invention comprises a first antibody reactive with mammalian α-BNP and a second antibody reactive with mammalian prepro-BNP or γ-BNP derivatives but not α-BNP, so as to determine specifically mammalian γ-BNP derivatives. Thus, an objective of the present invention is to avoid detection of peptide fragments not comprising the α-BNP moiety. According to the present inventors' findings, it is important to detect compounds that at least comprise structurally the α-BNP moiety in establishing an accurate method of diagnosing cardiac diseases, as described in the specification of the present case. The findings of the present inventors is neither described nor suggested in the Hunt et al. reference. Hunt et al. merely determined an ***N-terminal*** pro-BNP fragment [BNP(1-76)] irrespective of the fact that the fragment is accompanied with the α-BNP moiety and is not specific for a mammalian γ-BNP derivative.

Consequently, Hunt et al. does not disclose each and every limitation of the present inventive immunoassay as recited in the instant claims. Thus, the rejection should be withdrawn.

Response to 35 U.S.C. §103 Rejection

In item 5 on page 3, claims 6-8 were rejected under 35 U.S.C. §103 over Hunt et al. Applicants respectfully traverse this rejection.

The arguments proffered above in response to the previous rejection apply equally to the instant rejection.

As shown above, Hunt et al. does not disclose nor suggest the subject matter recited in claims 1-5. Claims 6-8 are drawn to a kit used for conducting the inventive immunoassays recited in claims 1-5. Accordingly, since the instant claims under rejection recite the subject matter of claims 1-5, which have been shown above to be novel and obvious over Hunt et al., then the instant claims are therefore patentable over the Hunt et al. reference

Consequently, the prior art of record does not teach or suggest the inventive immunoassay specific for a mammalian γ-BNP derivative as recited in the present claims. Therefore, all of the prior art rejections of record should be withdrawn in their entirety.

Conclusion

Accordingly, in view of the foregoing amendment and remarks, it is submitted that each ground of rejection set forth by the Examiner has been overcome and that the application is in condition for allowance.

A prompt Notice of Allowance is respectfully requested.

Respectfully submitted,

Hidehisa ASADA et al.

By: _____

Warren M. Cheek, Jr.
Registration No. 33,367
Attorney for Applicants

JJF/WMC/jlw
Washington, D.C. 20006-1021
Telephone (202) 721-8200
Facsimile (202) 721-8250
November 5, 2001

Part # 7

Version with Markings t Show Changes Made

1. (Amended) An immunoassay specific for a mammalian γ -BNP [derivatives] derivative, [characterized in that it] wherein said immunoassay comprises a [uses the] first antibody reactive with mammalian α -BNP and a [the] second antibody reactive with mammalian prepro-BNP or a γ -BNP [derivatives] derivative and not α -BNP.

2. (Amended) The immunoassay of claim 1, wherein [the] said mammalian γ -BNP [derivatives] derivative [comprise] comprises the amino acid sequence shown by the amino acid Nos. 27-[102] 134 of SEQ ID NO:[1] 2.

3. (Amended) The immunoassay of claim 1, wherein [the] said second antibody is [specific] reactive [for] with the amino acid sequence shown by [the] amino acid Nos. 27-102 of SEQ ID NO: [1] 2.

4. (Amended) The immunoassay of claim 1, wherein at least one of [the] said first and [the] said second antibodies is [detectably] labeled with a detectable label or is immobilized.

5. (Amended) The immunoassay of claim [1] 4, wherein [the] said detectable label is a radioactive isotope, an enzyme, a fluorescent substance, a luminescent substance[,] or a particle.

6. (Amended) A kit for immunoassay specific for a mammalian γ -BNP [derivatives] derivative [characterized in that it comprises the] comprising a first antibody reactive with mammalian α -BNP and a second antibody reactive with mammalian prepro-BNP or a γ -BNP [derivatives] derivative and not α -BNP.

7. (Amended) The kit of claim 6, wherein at least one of [the] said first and [the] said second antibodies is [detectably] labeled with a detectable label or is immobilized.

Version with Markings to Show Changes Made

DESCRIPTION

IMMUNOASSAY FOR BNP

This is a 371 of PCT/JP98/04063, filed September 10, 1998.

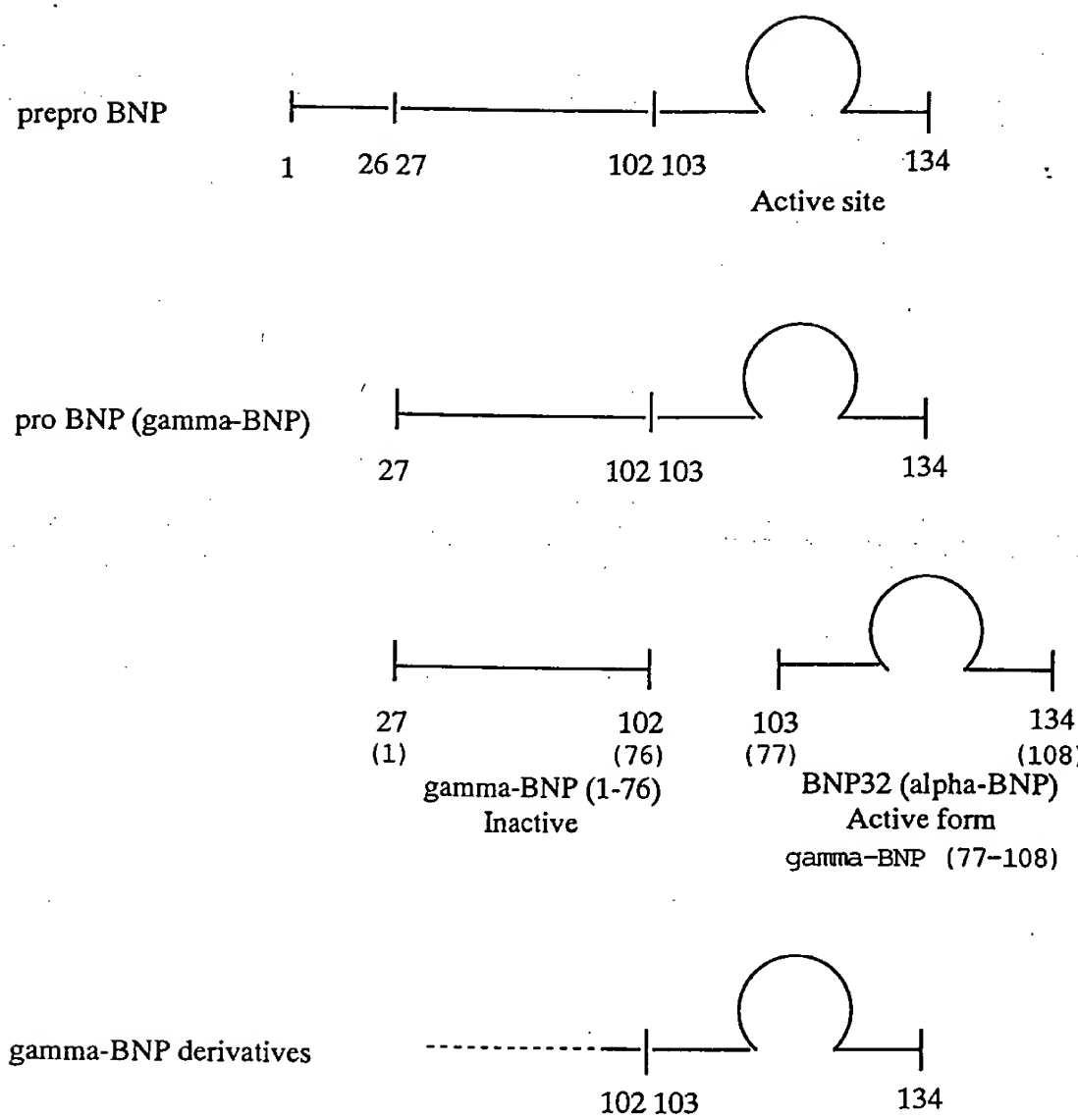
TECHNYCAL FIELD

The present invention relates to an immunoassay for the brain natriuretic peptide (BNP) which is a member of natriuretic peptide family, more specifically, it relates to an immunoassay for γ-BNP and derivatives thereof.

BACKGROUND ART

Natriuretic peptide family includes three members, i.e., atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and type C natriuretic peptide (CNP). Among them, ANP and BNP are cardiac hormones which are mainly biosynthesized in and secreted from the heart. ANP and BNP are similar in structure. ANP is a peptide of 28 amino acids with a ring (circular) structure formed by a disulfide bond between the 7th and the 23rd cysteine residues, while BNP is a peptide of 32 amino acids with a ring structure formed by a disulfide bond between the 10th and the 26th cysteine residues. These mature peptides of 28 and 32 amino acids have been considered to be produced from respective precursor when a leader sequence is cleaved off intracellularly or at the time of secretion. That is, there has been reported that human BNP is first synthesized as a preprohormone (hereinafter, referred to as prepro-BNP) in myocardial cells, which is split before or at the time of secretion between Ser^{26}-His^{27} to give pro-BNP (hereinafter, referred to as γ-BNP), and which is further split between Arg^{102}-Ser^{103} to give BNP-32 (hereinafter, referred to as α-BNP) and BNP(1-76), and that the former exhibits the activity. It

Appendix I


prepro BNP
1
26 27
102 103
134
Active site
pro BNP (gamma-BNP)
27
102 103
134
27
(1)
102
(76)
gamma-BNP (1-76)
Inactive
103
(77)
134
(108)
BNP32 (alpha-BNP)
Active form
gamma-BNP (77-108)
gamma-BNP derivatives
102 103
134